Remarks

The method as claimed now is for investigating blood or bone marrow for disseminated cancer cells (see page 5, lines 29–34, of the application).

Further, the genes have been defined more precisely. Thioredoxine reductase 1 is disclosed on page 16, line 32, and glutathione peroxidase 1 is disclosed on page 18, line 26, of the application. Moreover, manganese superoxide dismutase (MNSOD), thioredoxine reductase 1 (TXNRD1) and glutathione peroxidase 1 (GPX1) are the genes used in the exemplary embodiments described on pages 39 to 57 of the application.

Several new claims have been inserted which define the genes still more precisely. MNSODs, TXNRD1s and GPX1s of human origin are described on page 15, lines 9 and 10, page 17, lines 3 and 4, and page 18, lines 36 and 37, of the application. The MNSOD having the amino acid sequence SEQ ID NO:13, the TXNRD1 having the amino acid sequence SEQ ID NO:15, and the GPX1 having the amino acid sequence SEQ ID NO:17 are disclosed on page 15, lines 23–26, page 17, lines 12–14, and page 19, lines 9–11, of the application. Allelic variants thereof are described on page 15, lines 12–15, page 17, lines 6–10, and page 18, line 39, to page 19, line 7. The primer sequences are found on page 16, lines 17–19, page 18, lines 10–12, and page 20, lines 18–20.

Further, the method steps of previous claim 5 have been incorporated into claim 1, and it is now stated in claim 1 that an elevated expression of each gene determined in the cell-containing fraction as compared to its expression in the further cell-containing

fraction indicates the presence of disseminated cancer cells in the body fluid. See also under "evaluation" on page 28 of the application and previous claims 7 and 13.

Claim 4 as amended is now drawn to a particular embodiment of obtaining the cell-containing fraction from the body fluid with enrichment of cancer cells. This embodiment is disclosed on page 8, line 39, to page 9, line 5, of the application).

A new claim (new claim 25) has been added which is directed to mRNA determination (see page 14, lines 22–33, of the application.

Claim 1-4, 6 and 11-25 are now in the case. Claims 1-4, 6 and 11-17 are currently amended or previously presented. Claims 18-25 are new claims. Claims 9 and 10 which were withdrawn from further consideration, are cancelled.

We turn now to the objection to the specification. It is pointed out at page 3 of the office action, that "RNA" at page 40, line 14, 15 misspelled. Correction is made by amendment herein. Reconsideration is requested.

We turn now to the objections to the claims. Claim 1 is objected to because "I" is inconsistent with "ii" and "iii". Correction is made by amendment herein. Reconsideration is requested.

At pages 18 and 19 of the office action claims 1, 3 and 11 are rejected under 35 U.S.C. 102 (b) as anticipated by Seven et al. and Kizzki et al. Reconsideration is requested.

The method of amended claim 1 is characterized by a combination of the features defined in previous claims 1, 3, 4, 5, 7, and 13. The claimed subject matter of the amended claims is thus novel over the cited references.

As an aside, we note that the office action supposes that CuZnSOD is a manganese superoxide dismutase as defined in the present application. We disagree. The person of average skill in the art clearly distinguishes between a manganese superoxide dismutase (superoxide dismutase 2) and a CuZnSOD (superoxide dismutase 1). Although both enzymes catalyze the same process (i.e., the decomposition of superoxide radicals (O_2^-) to form hydrogen peroxide (H_2O_2) and belong to the same enzyme class 1.15.1.1 they are distinct enzymes. The assertion in the office action seems to be based on a misinterpretation of the paragraph-bridging pages 14 and 15 of the application. In this paragraph the reader is taught that the enzymes which constitute enzyme class 1.15.1.1 catalyze the decomposition of superoxide free radicals to form hydrogen peroxide and that such enzymes include manganese superoxide dismutase. This, however, does not mean that the manganese superoxide dismutases that are the subject of the invention include CuZnSOD.

We turn now to the rejection under 35 U.S.C. 112, first paragraph (lack of enablement) which begins at page 4 of the office action. Reconsideration is requested.

Enclosed please find a draft declaration by Professor Giesing, one of the two inventors. The declaration is relied on to rebut the lack of enablement rejection.

On pages 4 and 5, the office action acknowledges that the specification is enabling for a method which comprises steps (a) to (j) as defined on pages 4 and 5 of the office action. However, the office action asserts that the specification does not reasonably provide enablement for

i) the use of any body fluid from any species of organism,
ii) the absence of a reference sample,
iii) the use of any reference sample,
iv) determining the expression of any manganese superoxide dismutase gene, any thioredoxine reductase gene, or any glutathione peroxidase gene,
v) early diagnosis of a tumor, or
vi) estimating the risk to develop a metastatis or recurrence.

In response, consider the following:

The amended claims are drawn to a method for investigating blood or bone marrow for disseminated cancer cells. The specification teaches that in contrast to solid tumors, disseminated cancer cells are able to circulate in the body of an individual. This usually takes place via endogenous transport organs, especially body fluids, in particular blood (page 5, line 36 to page 6, line 4). The specification further teaches that disseminated cancer cells are expected to be present in blood and also bone marrow (page 5, lines 29–34). While the office action acknowledges that the method is enabling for blood it would be obvious to a person of average skill in the art that the method is also enabling for investigating bone marrow for disseminated cancer cells (see also Professor Giesing's declaration under item 5.4).

The claimed method now includes the use of a reference sample ("a further cell-containing fraction of the body fluid or of a comparable biological sample").

In the amended claims, the type of reference sample is defined as a further cell-containing fraction of **the** body fluid or of a comparable biological sample. Thus, the further cell-containing fraction is derived from the same body fluid as the cell-containing fraction or from a biological sample that is comparable to this body fluid. The specification teaches that the test principle is based on determining whether enrichment of cancer cells is associated with a measurable increase in MNSOD, TXNRD and GPX expression. The ratio of the expression measured in the test cell mixture to the expression

measured in the comparison cell mixture is therefore decisive (page 29, lines 33–39, of the application). It is against this background that the specification further teaches that it is sufficient for the method if the proportion of cancer cells is significantly higher in the test cell mixture than in the comparison cell mixture (page 29, lines 1–6, of the application). While the office action appears to acknowledge that the specification is enabling for the further cell-containing fraction being non-cancer cells derived from the body fluid of the individual whose body fluid is investigated for cancer cells, it will be obvious to a person of average skill in the art that 1) the comparison cell mixture may contain some proportions of cancer cells (as long as the proportion of cancer cells in the test cell mixture is sufficiently higher) and 2) the comparison cell mixture must not necessarily be derived from the same body fluid of the same individual as the test cell mixture. The office action further states that Seven et al. teach that constitutive levels and inducibility of antioxidant enzymes including superoxide dismutase and glutathione peroxidase vary for different tissues, and the expression of these enzymes may vary according to the type of cancer or tissue studies, resulting in controversy in the literature (see the bottom of page 16 of the office action). Seven et al. are concerned with antioxidant activity in blood plasma and erythrocytes. In contrast, the present invention is concerned with the determination of antioxidant enzymes in disseminated cancer cells. In blood, cancer cells are found in the mononuclear cell fraction which is different from the erythrocytes.

The amended claims now relate to the determination of genes of specific enzymes, i.e., manganese superoxide dismutase, thioredoxine reductase 1 and glutathione peroxidase 1. The office action asserts that the term "manganese superoxide dismutase"

includes copper-zinc superoxide dismutases. We disagree for the reasons already outlined above. While the office action further contends that different isoforms of MNSOD, TXNRD1 and GPX1 are expressed (page 16 of the office action), it acknowledges that the specification is enabling for the use of the specific primers and probes used in the exemplary embodiments described in the application (see pages 43 and 44 of the application). The applicants have checked which transcript variants are detected when these primers and probes are used. As stated in Professor Giesing's declaration, there are several transcript variants of MNSOD, TXNRD1 and GPX1 which will be amplified and thus detected when said primers and probes are used. This corroborates that the specification is enabling even when it comes to the detection of transcript variants. Moreover, it would be obvious to a person of ordinary skill in the art to use appropriate primer and probe designs if one of said variants is to be differentially detected.

The office action states that the specification does not teach the stage or grade of the cancers at the time blood was drawn and the investigations described in the exemplary embodiments carried out. It goes on to say that there is no indication that the cancer cells detected by increased expression of MNSOD, TXNRD1 or GPX1 are not a result of advanced metastatic cancer and the specification does not teach the sensitivity of the assay for early, non-metastatic cancer (see page 13, first full paragraph of the office action). The applicants maintain that the method of the present invention is suitable for the early diagnosis of cancer. However, in order to further the prosecution of the present application, claim 14 is now directed to the diagnosis of a tumor in general.

The office action asserts that the specification does not teach the classification of individuals as at risk or not at risk to develop a metastasis, or at risk to develop a recurrence (page 14, the end of the second full paragraph). Further, the office action refers to Pusztai and Hess, Shalan et al., Kroese et al., and Golub et al., asserting that the art demonstrates the unpredictable nature of extrapolating gene expression differences to a method of class prediction (see pages 14, 15 and 16 of the office action). Without providing a detailed discussion why the criticism described in said references does not apply to the assay of the present invention, it is safe to say that the method of the present invention is suitable for estimating the risk to develop a metastasis or a recurrence. The data described in Professor Giesing's declaration clearly corroborates that the method of the present invention is suitable for predicting not only the primary tumor but also relapse-free survival and the risk of developing distant or local relapse.

Allowance is requested

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